the design of liposomes for fusogenic delivery to OMV targets. There is therapeutic interest in delivery to Gram-negative OMV, e.g. where they exist in biofilm infections, or to elucidate a semi-synthetic approach to modify OMV lipid composition to generate useful delivery vehicles.

474-Pos Board B243
Macrophage Cell Fusion and Crosstalk with Myoblast Fusion
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Cell-to-cell fusion plays a pivotal role in various developmental processes, tissue homeostasis, immune response and, possibly, cancer. One of the key challenges in characterizing these complex and relatively slow membrane fusion events is to uncouple actual fusion stage from the preceding differentiation processes, which prepare the cells for fusion. Here we have focused on two very important and well characterized examples of cell-to-cell fusion, macrophage fusion that leads to osteoclast or giant cells formation and myoblast fusion in muscle development and regeneration. Proteins that mediate fusion stages of these processes remain obscure. Application of RANKL to RAW macrophage-like cells commits the cells to fusion with most fusion events to take place 72-96 hours later. We blocked this robust fusion by applying a reversible hemifusion inhibitor lysophosphatidylcholine (LPC) at 72 hours post-RANKL, and removed LPC at 88 hours. This approach has allowed us to accumulate the ready-to-fuse macrophages and then observe cell fusion events that would normally develop within 16h to develop within 30-90 min. Synchronization of cell fusion using LPC block has also worked for myoblast fusion. Antibodies against annexin V inhibited both macrophage fusion and myoblast fusion suggesting similarities between protein machineries involved in these fusion processes. We also found that co-incubation of fusion-committed RAW cells and primary myoblasts promotes myotube formation and inhibits osteoclast formation suggesting an intriguing cross-talk between these cells in the context of muscle regeneration as well as in inflammation & bone biology.

475-Pos Board B244
An Anionic Phospholipid Enables the Hydrophobic Surfactant Proteins to Alter Spontaneous Curvature
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The cationic, hydrophobic surfactant proteins, SP-B and SP-C (SPs), promote adsorption of vesicles to an air water interface and greatly increase the rate of forming an alveolar film. The available evidence suggests that the SPs may facilitate adsorption by promoting the generation of a negatively curved intermediate. The studies here tested whether the proteins could change the curvature of phospholipid leaflets in the inverse hexagonal (H_{II}) phase. Experiments with the uncharged dioleoyl phosphatidylethanolamine (DOPE) showed no change in the lattice-constant with added SPs. Pulmonary surfactant contains ~10% (mol:mol) anionic phospholipids. We therefore also examined mixtures of DOPE with 10% (mol:mol) of the anionic dioleoyl phosphatidylglycerol (DOPG) to investigate selective interactions with the SPs. Small angle X-ray scattering established the phase diagram (temperature - % protein) and lattice-constants for these lipids combined with the physiological mixture of the SPs. The hexagonal lattice-constant decreased linearly with increasing amounts of protein before reaching a temperature-dependent maximum change of 8-14% at ~1% (w:w) protein. To test whether this effect was strictly electrostatic, NaCl concentrations up to 3M provided electrostatic screening. The salt diminished but did not eliminate the dose-related change in the lattice-constant. Measurements at different hydrations showed that the separation between the pivotal plane and the aqueous core was unaffected by the proteins, indicating that the change in the lattice-constant produced by the proteins reflects a more negative spontaneous curvature. These results provide direct evidence that the hydrophobic surfactant proteins can enhance the negative curvature of lipid leaflets. Studies were conducted at the Stanford Synchrotron Radiation Lightsource.

476-Pos Board B245
Dynamics of the Influenza Hemagglutinin Fusion Peptide: A Comparison of MD and NMR
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Membrane insertion of influenza hemagglutinin (HA) is essential for viral membrane fusion. Previous NMR relaxation studies on bicelles [1, 2] analyzed the 23 conserved N-terminal residues of the HA2 subunit (fusion peptide). It was shown that this peptide, which adopted a helical hairpin structure, experiences wobbling motions relative to the bilayer surface on the ns timescale. Here, the dynamics of the HA fusion peptide hairpin on a DMPC membrane are studied using molecular dynamics (MD) simulations. Simulations were performed with the acidic groups (E11 and D19) protonated and unprotonated. Internal correlation functions of backbone N-H vectors are determined over the 100-ns MD simulations, and are fit to the Lipari-Szabo model free approach [3]. The calculated order parameters and correlation times are similar to those determined experimentally. Starting from an initial orientation parallel to the membrane, during the simulations the hairpin rotated nearly 90° around the axis that is parallel to the two helices, with the N-terminal helix buried more deeply in the lipid tail region.
[1] J. L. Lorieau, J. M. Louis, and A. Bax, Proceedings of the National Academy of Sciences of the United States of America 107 (2010) 11341.
[2] J. L. Lorieau, J. M. Louis, and A. Bax, Journal of the American Chemical Society 133 (2011) 14184.
[3] G. Lipari and A. Szabo, Biophysical Journal 37 (1982) A380.

477-Pos Board B246
Lipid Bilayer Curvature Frustration
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The free energy of bending of surfaces composed of biological lipids is derived, computed, and compared with experiment. Simulations of two distinct systems, one a planar bilayer, the other the inverse hexagonal phase indicate consistent mechanical properties and curvature preferences, with DOPE having a spontaneous curvature, R0 = −26 Angstroms and DOPC preferring to be approximately flat (R0=-65 Angstroms). Additionally, a well-defined pivotal plane, where a DOPE leaflet bends at constant area, has been determined to be near the glycerol region of the lipid, consistent with the experimentally predicted plane. By examining the curvature frustration of both high and low curvature, the transferability of experimentally determined bending constants is supported. How to predict the effects of biologically active molecules on the mechanical properties of lipid bilayers under well-controlled conditions will be examined.

478-Pos Board B247
Mechansim of the Early Stages of the Lamellar/Bicontinuous Cubic Phase Transition
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The transition between lamellar and bicontinuous inverted cubic (Q_{II}) phases is mediated by catenoidal bilayer channels, which are also the structures that are produced by membrane fusion. The Q_{II} phase forms by production of these channels in an array of initially flat bilayers. As the number of channels increase with increasing temperature, they initially form a disordered, metastable array in the lamellar phase, which has been referred to as the ''isotropic phase.'' The isotropic phase accomplishes the topological change between the lamellar and Q_{II} phases. The disordered lattice subsequently transforms into the Q_{II} phase by local bending of the bilayers. There are discordant observations concerning the rate of formation and temperature interval of existence for the ''isotopic phase.'' Here, the expected dimensions of channels are predicted as a function of temperature, as well as the extent of ''isotropic phase'' formation as a function of temperature and sample water content. The predictions are made using the fourth order curvature energy model previously used to rationalize the stability of the Q_{II} phase in phospholipids (Langmuir, 2010, **26**:8673). The extent of channel and ''isotropic phase'' formation is sensitive to the interaction energy of the flat bilayers in the lamellar phase, the lateral dimensions of the lamellar phase bilayers and the sample water volume fraction, as well as the temperature and curvature elastic parameters of the lipid. The theory, as well as the hysteretic nature of the transition process, accounts for the apparent conflict between early observations, made mostly by phosphorus NMR.

479-Pos Board B248
Calculating Minimal Energy Shapes of Fusion Pores
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We have developed a new computational model to calculate the shape of fusion pores that are in a state of minimal elastic energy as a function of pore length and lumen radius. A Helfrich Hamiltonian accounting for splay and lipid tilt was used for calculations. Minimal-energy shapes were obtained by numerically solving steepest descent partial differential equations derived from the Hamiltonian. The energy landscape was calculated by describing the bilayer as a single surface–the midplane between monolayers–or by describing the